We Claim:

5

10

15

- 1. A mobility-modifying cyanine dye comprising: (i) a first substituted or unsubstituted parent heteroaromatic benzazole/benzazolium ring system having a linking moiety of the formula –L–LG, where L is a linker and LG is a linking group, attached to the heteroaromatic ring nitrogen; (ii) a second substituted or unsubstituted parent heteroaromatic benzazole/benzazolium ring system having a mobility-modifying moiety attached to the heteroaromatic ring nitrogen; and an electron delocalizing bridge connecting the first and second parent benzazole/benzazolium rings *via* their respective C2 carbons, wherein when said mobility-modifying moiety has a net charge of -2 or less or +1 or greater.
- 2. The mobility-modifying cyanine dye of Claim 1 in which the bridge is a compound selected from the group consisting of:

(B.1)
$$-(CR^1=CR^2)_k-(CR^3=CR^4)_r-(CR^5-CR^6)_m-CR^7=$$

and

(B.2)
$$R^{10}$$
 R^{10} R^{7} $R^{10'}$

wherein:

k, l, and m are each independently integers from 0 to 1;

 R^1 , R^2 , R^3 , R^4 , R^5 , R^6 and R^7 are each independently selected from the group consisting of hydrogen, halogen, -F, -Cl, -CN, $-CF_3$, (C_1-C_6) alkyl, (C_5-C_{14}) aryl or 5-14 membered heteroaryl;

R¹⁰ and R¹⁰ are each independently selected from the group consisting of hydrogen, oxygen, halogen, -F, -Cl, -CN, -CF₃, -OR, -SR, -NRR, (C₁-C₆) alkyl, (C₅-C₁₄) aryl or 5-14 membered heteroaryl, where each R is independently hydrogen or (C₁-C₆) alkyl; and

5

p is an integer from 0 to 2, where in structural formula (B.2), the dotted lines at substituents R¹⁰ represent bonds that may be independently either single bonds or a double bonds, depending upon the identities of the substituents.

10

- 3. The mobility-modifying cyanine dye of Claim 2 in which the bridge is a compound of structural formula (B.1).
- 4. The mobility-modifying cyanine dye of Claim 3 in which the sum of k, land m is 2.

15

5. The mobility-modifying cyanine dye of Claim 2 in which the bridge is a compound of structural formula (B.1) wherein R¹, R², R³, R⁴, R⁵, R⁶ and R⁷ are each hydrogen.

20

6. The mobility-modifying cyanine dye of Claim 1 in which the bridge is -CH=CH-CH=CH-CH=.

7. The mobility-modifying cyanine dye of Claim 1 in which the mobilitymodifying moiety has a net positive charge.

25

- 8. The mobility-modifying cyanine dye of Claim 1 in which the mobilitymodifying moiety has a net negative charge.
- 9. The mobility-modifying cyanine dye of Claim 1 in which the mobilitymodifying moiety has the structure:

$$R^{28}$$
 R^{24}
 R^{27}
 R^{26}

or a salt thereof, wherein:

5

n is an integer from 1 to 6 (preferably 1 to 3);

 R^{24} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{25} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups;

10

 R^{25} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{24} or R^{26} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups;

15

 R^{26} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{25} or R^{27} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups; and

20

 R^{27} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{26} or R^{28} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups; and

 R^{28} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{27} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups.

10. The mobility-modifying cyanine dye of Claim 1 in which the mobility-modifying moiety has the structure:

$$\begin{array}{c}
R^{29} \\
\downarrow \\
o \\
R^{30}
\end{array}$$

or a salt thereof, wherein:

o is an integer from 1 to 3;

q is an integer from 1 to 3;

 R^{29} is a strong anionic substituent, $-S(O)_2O^-$ or $-O-S(O)_2O^-$,

each R^{30} is independently selected from the group consisting of hydrogen a strong anionic substituent, $-S(O)_2O^-$ and $-O-S(O)_2O^-$; and

 R^{31} is selected from the group consisting of hydrogen, a strong anionic substitutent, $-S(O)_2O^-$, $-O-S(O)_2O^-$ and $-CH_3$,

with the proviso that MM has a net charge of at least -2 at a pH in the range of about pH 6 to pH 10.

- 11. The mobility-modifying cyanine dye of Claim 1 in which the first and second heteroaromatic benzazole/benzazolium ring systems are the same.
- 12. The mobility-modifying cyanine dye of Claim 1 in which the first parent heteroaromatic benzazole/benzazolium ring system has the structure:

20

15

5

or a salt thereof, wherein:

5

Z is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹-, where R^8 and R^9 when taken alone, are each independently (C_1 - C_6) alkyl, or when taken together are (C_4-C_5) alkyleno or (C_4-C_5) alkano;

10

15

20

R¹¹, R¹², R¹³ and R¹⁴, when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C_5-C_6) arylaryl, (C_5-C_6) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroarylheteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroarylheteroalkyl independently substituted with one or more W.

or when taken together with an adjacent Rn are each independently selected from the group consisting of (C₆-C₁₀) aryleno, (C₆-C₁₀) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W; and

each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C_1-C_6)

perhaloalkyl, $-CX_3$, -CN, -OCN, -SCN, -NO, $-NO_2$, $=N_2$, $-N_3$, -NHOH, $-S(O)_2R$, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', and -C(NR)NRR, wherein:

each X is independently a halogen;

each R is independently -H, -NR"R", -C(O)R", -S(O₂)R", (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

each R' is independently (C_1 - C_6) alkyl, (C_1 - C_6) alkanyl, (C_2 - C_6) alkenyl and (C_2 - C_6) alkynyl, (C_5 - C_{10}) aryl, (C_6 - C_{16}) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each R" is independently -H, (C_1 - C_6) alkyl, (C_1 - C_6) alkanyl, (C_2 - C_6) alkynyl, (C_5 - C_{10}) aryl, (C_6 - C_{16}) arlyalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl.

13. The mobility-modifying cyanine dye of Claim 1 in which the second parent heteroaromatic benzazole/benzazolium ring system has the structure:

$$R^{20}$$

$$R^{21}$$

$$R^{22}$$

$$R^{22}$$

or a salt thereof, wherein:

20

25

5

10

15

Z' is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹, where R⁸ and R⁹, when taken alone, are each independently (C_1 - C_6) alkyl, or when taken together are (C_4 - C_5) alkyleno or (C_4 - C_5) alkano;

 R^{19} , R^{20} , R^{21} and R^{22} , when taken alone, are each independently selected from the group consisting of hydrogen, (C_1 - C_6) alkyl, (C_1 - C_6) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl

independently substituted with one or more W, (C_5-C_{10}) aryl, (C_5-C_{10}) aryl independently substituted with one or more W, (C_5-C_6) arylaryl, (C_5-C_6) arylaryl independently substituted with one or more W, (C_6-C_{16}) arylalkyl, (C_6-C_{16}) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

or when taken together with an adjacent R^n are each independently selected from the group consisting of (C_6-C_{10}) aryleno, (C_6-C_{10}) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W; and

each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C_1-C_6) perhaloalkyl, -CX₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -NHOH, -S(O)₂R, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(S)SR', and -C(NR)NRR, wherein:

each X is independently a halogen;

5

10

15

20

25

30

each R is independently -H, -NR"R", -C(O)R", -S(O₂)R", (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

each R' is independently (C_1 - C_6) alkyl, (C_1 - C_6) alkanyl, (C_2 - C_6) alkenyl and (C_2 - C_6) alkynyl, (C_5 - C_{10}) aryl, (C_6 - C_{16}) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each R" is independently -H, (C_1 - C_6) alkyl, (C_1 - C_6) alkanyl, (C_2 - C_6) alkynyl, (C_5 - C_{10}) aryl, (C_6 - C_{16}) arlyalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl.

14. A mobility-modifying cyanine dye according to Claim 1 in which the first and second heteroaromatic benzazole/benzazolium rings are each the same or different substituted or unsubstituted indoline/indolinium ring.

15. The mobility-modifying cyanine dye of Claim 1 which has the structure:

$$R^{13}$$
 R^{14}
 R^{13}
 R^{14}
 R^{14}
 R^{15}
 R^{15}
 R^{15}
 R^{19}
 R^{10}
 R^{10}

5

10

15

or a salt thereof, wherein:

k, l, and m are each independently integers from 0 to 1;

 R^1 , R^2 , R^3 , R^4 , R^5 , R^6 and R^7 are each independently selected from the group consisting of hydrogen, halogen, -F, -Cl, -CN, $-CF_3$, (C_1-C_6) alkyl, (C_5-C_{14}) aryl and 5-14 membered heteroaryl;

MM is a mobility-modifying moiety;

L is a linker;

 R_x is a reactive functional group;

Z is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹-, where R⁸ and R⁹ when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkyleno or (C₄-C₅) alkano;

Z' is selected from the group consisting of -S-, -O-, -Se- and -CR⁸'R⁹', where R⁸'and R⁹', when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkyleno or (C₄-C₅) alkano;

20

 R^{11} , R^{12} , R^{13} and R^{14} , when taken alone, are each independently selected from the group consisting of hydrogen, (C_1 - C_6) alkyl, (C_1 - C_6) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C_5 - C_{10}) aryl, (C_5 - C_{10}) aryl independently substituted with one or more W, (C_5 - C_6) arylaryl, (C_5 - C_6) arylaryl independently

substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroarylheteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroarylheteroalkyl and 6-16 membered heteroarylheteroalkyl independently substituted with one or more W,

or when taken together with an adjacent R^n are each independently selected from the group consisting of (C_6-C_{10}) aryleno, (C_6-C_{10}) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

 R^{19} , R^{20} , R^{21} and R^{22} , when taken alone, are each independently selected from the group consisting of hydrogen, (C_1-C_6) alkyl, (C_1-C_6) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C_5-C_{10}) aryl, (C_5-C_{10}) aryl independently substituted with one or more W, (C_5-C_6) arylaryl, (C_5-C_6) arylaryl independently substituted with one or more W, (C_6-C_{16}) arylalkyl, (C_6-C_{16}) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

or when taken together with an adjacent R^n are each independently selected from the group consisting of (C_6-C_{10}) aryleno, (C_6-C_{10}) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C_1-C_6)

5

10

15

20

perhaloalkyl, $-CX_3$, -CN, -OCN, -SCN, -NO, $-NO_2$, $=N_2$, $-N_3$, -NHOH, $-S(O)_2R$, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', and -C(NR)NRR, wherein:

each X is independently a halogen;

5

10

15

each R is independently -H, -NR"R", -C(O)R", -S(O₂)R", (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

each R' is independently (C_1 - C_6) alkyl, (C_1 - C_6) alkanyl, (C_2 - C_6) alkenyl and (C_2 - C_6) alkynyl, (C_5 - C_{10}) aryl, (C_6 - C_{16}) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each R" is independently -H, (C_1 - C_6) alkyl, (C_1 - C_6) alkanyl, (C_2 - C_6) alkynyl, (C_5 - C_{10}) aryl, (C_6 - C_{16}) arlyalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl.

- 16. The mobility-modifying cyanine dye of Claim 15 in which Z is $-NR^8R^9$, where R^8 and R^9 are each independently (C_1 - C_6) alkano; and Z' is $-NR^8R^9$, where R^8 and R^9 are each independently (C_1 - C_6) alkano.
- 17. The mobility-modifying cyanine dye of Claim 15 which is selected from the group consisting of:

(I.D)
$$R^{13}$$
 R^{14} R^{19} R^{20} R^{12} R^{11} R^{20} R^{22} R^{21} R^{21} R^{20} R^{20} R^{21} R^{21} R^{21} R^{21} R^{21} R^{21} R^{21} R^{21} R^{21}

(I.E)
$$R^{32}$$
 R^{33} R^{34} R^{35} R^{34} R^{35} R^{30} R^{30} R^{12} R^{11} R^{11} R^{22} R^{21} R^{21} R^{22} R^{21} R^{21} R^{22} R^{21} R^{21} R^{22} R^{21} R^{21} R^{22} R^{21} R^{22} R^{21} R^{21} R^{22} R^{21} R^{22} R^{21} R^{21} R^{22} R^{21} R^{21} R^{22} R^{21} R^{22} R^{21} R^{2

(I.F)
$$R^{32}$$
 R^{33} R^{19} R^{20} R^{12} R^{11} R^{20} R^{22} R^{21} R^{21} R^{20} R^{22} R^{21} R^{21} R^{20} R^{22} R^{21}

5

(I.G)
$$R^{13}$$
 R^{14} R^{35} R^{36} R^{36} R^{12} R^{11} R^{11} R^{22} R^{21} R^{22} R^{22} R^{21} R^{22} R^{22} R^{21} R^{22} R^{21} R^{22} R^{21} R^{22} R^{21} R^{21} R^{22} R^{22} R^{21} R^{22} R^{21} R^{22} R^{21} R^{22} R^{22} R^{22} R^{22} R^{23} R^{22} R^{23} R^{23} R^{23} R^{2

(I.H)
$$R^{13}$$
 R^{14} R^{19} R^{20} R^{20} R^{21} R^{21} R^{21} R^{20} R^{21} R^{20} R^{21} R^{2

(I.I)
$$R^{31}$$
 R^{32} R^{33} R^{34} R^{35} R^{53} R^{34} R^{35} R^{53} R^{30} R^{12} R^{11} R^{11} R^{22} R^{21} R^{21} R^{22} R^{21} R^{22} R^{21} R^{22} R^{21}

(I.J)
$$R^{32}$$
 R^{33} R^{19} R^{20} R^{12} R^{11} R^{20} R^{22} R^{21} R^{21} R^{20} R^{22} R^{21}

(I.K) R^{13} R^{14} R^{14} R^{15} R^{36} R^{36} R^{12} R^{11} R^{12} R^{11} R^{22} R^{21} R^{21} R^{21} R^{21} R^{21} R^{21} R^{21} R^{21} R^{21} R^{21}

10

5

or a salt thereof, wherein:

 R^{11} , R^{12} , R^{13} , R^{14} , R^{19} , R^{20} , R^{21} , R^{22} , R^{30} , R^{31} , R^{32} , R^{33} , R^{34} , R^{35} , R^{36} and R^{37} are each independently selected from the group consisting of hydrogen, $-S(O)_2O^-$ and $-O-S(O)_2O^-$.

18. A labeled nucleoside/tide or nucleoside/tide analog having the structure:

5

10

15

20

or a salt thereof, wherein:

D is a mobility-modifying cyanine dye chromophore;

L is a first linker which is attached to D at a heteroaromatic ring nitrogen;

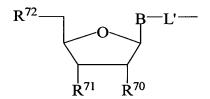
R⁴¹ is a covalent linkage;

NUC is a nucleoside/tide or nucleoside/tide analog; and

L' is a second linker which is attached to the nucleobase or sugar moiety of

NUC.

- 19. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 which is enzymatically incorporable.
- 20. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 which is a terminator.
- 21. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 which is enzymatically extendable.
 - 22. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 in which -L'-NUC taken together has the structure:



or a salt thereof, wherein:

B is a nucleobase;

L' is (C_1-C_{20}) alkyldiyl, (C_1-C_{20}) alkyleno, (C_2-C_{20}) alkyno, (C_2-C_{20}) alkeno 2-20 membered heteroalkyldiyl, 2-20 membered heteroalkyleno, 2-20 membered heteroalkyno or 2-20 membered heteroalkeno;

R⁷⁰ and R⁷¹, when taken alone, are each independently selected from the group consisting of hydrogen, hydroxyl and a moiety which blocks polymerase-mediated template-directed polymerization, or when taken together form a bond such that the illustrated sugar is 2',3'-didehydroribose; and

R⁷² is selected from the group consisting of hydroxyl, a phosphate ester

phosphate ester analog.

23. The labeled nucleoside/tide or nucleoside/tide analog of Claim 22 in which L' is selected from the group consisting of:

-C≡C−CH₂−, where the terminal sp carbon is covalently attached to nucleobase B and the terminal methylene (sp^3) carbon is covalently attached to R⁴¹; and -C≡C−CH₂−O−CH₂−CH₂−NR⁴⁷−R⁴⁸−, where R⁴⁷ is hydrogen or (C₁-C₆) alkyl and R⁴⁸ is −C(O)−(CH₂)_r−, −C(O)−CHR⁴⁹−, −C(O)−C≡C−CH₂− or −C(O)− φ −(CH₂)_r−, where each r is independently an integer from 1 to 5 and φ is C₆ aryldiyl or 6-membered heteroaryldiyl and R⁴⁹ is hydrogen, (C₁-C₆) alkyl or a side chain of an encoding or non-encoding amino acid, and where the terminal sp carbon is covalently attached to nucleobase B and the other terminal group is covalently attached to R⁴¹.

24. The labeled nucleoside/tide or nucleoside/tide analog of Claim 22 in which nucleobase B is a purine, a 7-deazapurine, a pyrimidine, a normal nucleobase or a common

5

10

15

analog of a normal nucleobase.

25. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 in which D has the structure:

 R^{14} R^{13} R^{14} R^{19} R^{13} R^{14} R^{19} R^{20} R^{19} R^{20} R^{20}

or a salt thereof, wherein:

k, l, and m are each independently integers from 0 to 1;

 R^1 , R^2 , R^3 , R^4 , R^5 , R^6 and R^7 are each independently selected from the group consisting of hydrogen, halogen, -F, -Cl, -CN, $-CF_3$, (C_1-C_6) alkyl, (C_5-C_{14}) aryl or 5-14 membered heteroaryl;

MM is a mobility-modifying moiety;

Z is selected from the group consisting of -S-, -O-, -Se- and -CR 8 R 9 -, where R 8 and R 9 when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkyleno or (C₄-C₅) alkano;

Z' is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R^{9'}, where R^{8'}and R^{9'}, when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkyleno or (C₄-C₅) alkano;

 R^{11} , R^{12} , R^{13} and R^{14} , when taken alone, are each independently selected from the group consisting of hydrogen, (C_1 - C_6) alkyl, (C_1 - C_6) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C_5 - C_{10}) aryl, (C_5 - C_{10}) aryl independently

5

15

substituted with one or more W, (C_5-C_6) arylaryl, (C_5-C_6) arylaryl independently substituted with one or more W, (C_6-C_{16}) arylalkyl, (C_6-C_{16}) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroarylheteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroarylheteroalkyl and 6-16 membered heteroarylheteroalkyl independently substituted with one or more W,

10

5

or when taken together with an adjacent R^n are each independently selected from the group consisting of (C_6-C_{10}) aryleno, (C_6-C_{10}) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

R¹⁹, R²⁰, R²¹ and R²², when taken alone, are each independently selected

15

from the group consisting of hydrogen, (C_1-C_6) alkyl, (C_1-C_6) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C_5-C_{10}) aryl, (C_5-C_{10}) aryl independently substituted with one or more W, (C_5-C_6) arylaryl, (C_5-C_6) arylaryl independently substituted with one or more W, (C_6-C_{16}) arylalkyl, (C_6-C_{16}) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-6 membered heteroarylheteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroarylheteroalkyl and 6-16 membered heteroarylheteroalkyl independently substituted with one or more W, 6-16 membered heteroarylheteroalkyl independently substituted with one or more W, 6-16 membered heteroarylheteroalkyl independently substituted with one or more W, 6-16 membered heteroarylheteroalkyl independently substituted with one or more W, 6-16 membered heteroarylheteroalkyl independently substituted with one or more W,

25

20

or when taken together with an adjacent R^n are each independently selected from the group consisting of (C_6-C_{10}) aryleno, (C_6-C_{10}) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C_1-C_6) perhaloalkyl, -CX₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -NHOH, -S(O)₂R, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(S)SR', and -C(NR)NRR, wherein:

each X is independently a halogen;

each R is independently -H, -NR"R", -C(O)R", -S(O₂)R", (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl;

each R' is independently (C_1 - C_6) alkyl, (C_1 - C_6) alkanyl, (C_2 - C_6) alkenyl and (C_2 - C_6) alkynyl, (C_5 - C_{10}) aryl, (C_6 - C_{16}) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each R" is independently -H, (C_1 - C_6) alkyl, (C_1 - C_6) alkanyl, (C_5 - C_{10}) aryl, (C_6 - C_{16}) arlyalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

the dotted line at the heteroaromatic ring nitrogen indicates the point of attachment of linker L.

26. A mobility-modifying phosphoramidite reagent having the structure:

(III)
$$R^{62}$$
 $N-P-O-L"-R^{41}-L-D$ R^{60}

or a salt thereof, wherein:

N, O and P are nitrogen, oxygen and phosphorous, respectively;

D is a mobility-modifying dye chromophore or a protected derivative thereof;

L is a first linker;

R⁴¹ is a bond or a covalent linkage;

L" is a bond or a second linker;

25

20

5

10

15

-135-

R⁶⁰ is a phosphite ester protecting group;

 R^{61} , when taken alone, is selected from the group consisting of (C_1-C_6) alkyl, (C_1-C_6) alkanyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, (C_3-C_{10}) cycloalkyl, (C_5-C_{20}) aryl and (C_6-C_{26}) arylalkyl, or when taken together with R^{62} forms a straight-chain or branched (C_2-C_{10}) alkyleno or a straight-chain or branched 2-10 membered heteroalkyleno; and

 R^{62} , when taken alone, is selected from the group consisting of (C_1-C_6) alkyl, (C_1-C_6) alkanyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, (C_3-C_{10}) cycloalkyl, (C_5-C_{20}) aryl and (C_6-C_{26}) arylalkyl, or when taken together with R^{61} forms a straight-chain or branched (C_2-C_{10}) alkyleno or a straight-chain or branched 2-10 membered heteroalkyleno.

10

5

27. The mobility-modifying phosphoramidite reagent according to Claim 26 which has the structure:

15
$$\begin{array}{c} R^{63} - O \\ R^{62} \\ N - P - O \\ R^{61} \\ O \\ R^{60} \end{array}$$
 (III.A)

wherein:

20

N, P and O are nitrogen, phosphorous and oxygen, respectively; R^{41} , L, D, R^{60} , R^{61} and R^{62} are as previously defined; R^{63} is hydrogen or an acid-labile hydroxyl protecting group; and ν is an integer from 1 to 30.

28. A mobility-modifying phosphoramidite reagent having the structure:

(IV) $R^{63} - O - B - L' - R^{41} - L - D$

or a salt thereof, wherein:

O, P and N are oxygen, phosphorous and nitrogen, respectively;

B is a nucleobase or a protected derivative thereof;

D is a mobility-modifying dye chromophore or a protected derivative thereof;

L is a first linker which is attached to D at a heteroaromatic ring nitrogen; R⁴¹ is a bond or a covalent linkage;

L' is a bond or a second linker;

R⁶⁰ is a phosphite ester protecting group;

 R^{61} , when taken alone, is selected from the group consisting of (C_1-C_6) alkyl, (C_1-C_6) alkanyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, (C_3-C_{10}) cycloalkyl, (C_5-C_{20}) aryl and (C_6-C_{26}) arylalkyl, or when taken together with R^{62} forms a straight-chain or branched (C_2-C_{10}) alkyleno or a straight-chain or branched 2-10 membered heteroalkyleno; and

 R^{62} , when taken alone, is selected from the group consisting of (C_1-C_6) alkyl, (C_1-C_6) alkanyl, (C_2-C_6) alkenyl, (C_2-C_6) alkynyl, (C_3-C_{10}) cycloalkyl, (C_5-C_{20}) aryl and (C_6-C_{26}) arylalkyl, or when taken together with R^{61} forms a straight-chain or branched (C_2-C_{10}) alkyleno or a straight-chain or branched 2-10 membered heteroalkyleno; and

R⁶³ is hydrogen or an acid-labile hydroxyl protecting group

25

5

10

15

29. The mobility-modifying phosphoramidite reagent according to Claim 26, 27 or 28 in which D has the structure:

$$R^{13}$$
 R^{14}
 R^{13}
 R^{14}
 R^{19}
 R^{10}
 R^{10}

or a salt thereof, wherein:

5

10

15

20

k, l, and m are each independently integers from 0 to 1;

 R^1 , R^2 , R^3 , R^4 , R^5 , R^6 and R^7 are each independently selected from the group consisting of hydrogen, halogen, -F, -Cl, -CN, $-CF_3$, (C_1-C_6) alkyl, (C_5-C_{14}) aryl or 5-14 membered heteroaryl;

MM is a mobility-modifying moiety;

Z is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹-, where R⁸ and R⁹ when taken alone, are each independently (C_1-C_6) alkyl, or when taken together are (C_4-C_5) alkyleno or (C_4-C_5) alkano;

Z' is selected from the group consisting of -S-, -O-, -Se- and -CR⁸'R⁹', where R⁸'and R⁹', when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkyleno or (C₄-C₅) alkano;

 R^{11} , R^{12} , R^{13} and R^{14} , when taken alone, are each independently selected from the group consisting of hydrogen, (C_1 - C_6) alkyl, (C_1 - C_6) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C_5 - C_{10}) aryl, (C_5 - C_{10}) aryl independently substituted with one or more W, (C_5 - C_6) arylaryl, (C_5 - C_6) arylaryl independently substituted with one or more W, (C_6 - C_{16}) arylalkyl, (C_6 - C_{16}) independently substituted with

one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroarylheteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroarylheteroalkyl and 6-16 membered heteroarylheteroalkyl independently substituted with one or more W,

5

10

15

20

25

30

or when taken together with an adjacent R^n are each independently selected from the group consisting of (C_6-C_{10}) aryleno, (C_6-C_{10}) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

R¹⁹, R²⁰, R²¹ and R²², when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

or when taken together with an adjacent R^n are each independently selected from the group consisting of (C_6-C_{10}) aryleno, (C_6-C_{10}) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C_1-C_6) perhaloalkyl, -CX₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -NHOH, -S(O)₂R, -C(O)R,

-C(S)R, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', and -C(NR)NRR, wherein:

each X is independently a halogen;

5

10

15

20

each R is independently -H, -NR"R", -C(O)R", -S(O₂)R", (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl;

each R' is independently (C_1 - C_6) alkyl, (C_1 - C_6) alkanyl, (C_2 - C_6) alkenyl and (C_2 - C_6) alkynyl, (C_5 - C_{10}) aryl, (C_6 - C_{16}) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each R" is independently -H, (C_1 - C_6) alkyl, (C_1 - C_6) alkanyl, (C_2 - C_6) alkynyl, (C_5 - C_{10}) aryl, (C_6 - C_{16}) arlyalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

the dotted line at the heteroaromatic ring nitrogen indicates the point of attachment of linker L.

30. The mobility-modifying phosphoramidite reagent of Claim 29 in which MM has the structure:

$$R^{28}$$
 R^{27}
 R^{26}
 R^{25}

or a salt thereof, wherein:

n is an integer from 1 to 6 (preferably 1 to 3);

 R^{24} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{25} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups;

 R^{25} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{24} or R^{26} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups;

5

 R^{26} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{25} or R^{27} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups; and

10

 R^{27} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{26} or R^{28} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups; and

15

 R^{28} , when taken alone, is hydrogen, a strong anionic substituent, $-S(O)_2O^-$, or $-O-S(O)_2O^-$, or when taken together with R^{27} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-S(O)_2O^-$, or $-O-S(O)_2O^-$ groups.

20

31. The mobility-modifying phosphoramidite reagent according to Claim 26, 27 or 28 in which L is -CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-(pentano).

25

- 32. The mobility-modifying phosphoramidite reagent according to Claim 26, 27 or 28 in which R^{41} is a covalent linkage formed upon the reaction between an electrophile and a nucleophile.
- 33. The mobility-modifying phosphoramidite reagent according to Claim 31 in which R^{41} has the structure $-C(O)-NR^{56}$, where R^{56} is hydrogen or (C_1-C_6) alkyl.
- 34. A polynucleotide labeled with a mobility-modifying dye according to Claim 1.

35. A method of generating a labeled primer extension product, comprising the step of enzymatically extending a primer-target hybrid in the presence of a mixture of enzymatically-extendable nucleotides capable of supporting continuous primer extension and a terminator, wherein said primer or said terminator is labeled with a mobility-modifying dye according to Claim 1.

5

10

15

20

25

- 36. The method of Claim 35 in which the terminator is a mixture of four different terminators, one which terminates at a template A, one which terminates at a template G, one which terminates at a template C and one which terminates at a template T or U and wherein at least one of the terminators is labeled with a mobility-modifying dye according to Claim 1.
- 37. The method of Claim 36 in which each of the four different terminators is labeled with a different, spectrally-resolvable fluorophore, and one of the terminators is selected from the group consisting of Compound 29 and Compound 32.
- 38. A kit for generating a labeled primer extension product, comprising enzymatically-extendable nucleotides capable of supporting continuous primer extension and a terminator, wherein said primer or said terminator is labeled with a mobility-modifying cyanine dye according to Claim 1.
- 39. The kit of Claim 38 in which the terminator is a set of four different mobility-modified terminators, one which terminates at a template A, one which terminates at a template C and one which terminates at a template T or U.
- 40. The kit of Claim 39 in which the set of four different terminators is a set of mobility-matched terminators.
 - 41. The kit of Claim 39 in which the set of mobility-matched terminators

Our Case No. 4460D1

comprises Compounds 31, 32, 33 and 34.